(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

B5

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 March 2004 (04.03.2004)

PCT

(10) International Publication Number
WO 2004/017950 A2

(51) International Patent Classification[7]: A61K 31/00, 31/437, 31/519, A61P 31/12, 31/18

(21) International Application Number: PCT/GB2003/003676

(22) International Filing Date: 22 August 2003 (22.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/405,548 22 August 2002 (22.08.2002) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/017950 A2

(54) Title: PHOSPHADIDYLINOSITOL 3,5-BIPHOSPHATE INHIBITORS AS ANTI-VIRAL AGENTS

(57) Abstract: Phosphatidylinositol 3,5-biphosphate signaling has been found to be critical to the process of viral sorting, traffiking and viral budding from mammalian cells. Inhibitors of the phosphatidylinositol 3,5-biphosphate pathway therefore have activity as anti-viral agents.

PHOSPHATIDYLINOSITOL-3,5-BIPHOSPHATE INHIBITORS AS ANTI-VIRAL AGENTS

Field of the Invention

The present invention relates to compounds which inhibit the phosphatidylinositol 3,5-biphosphate pathway and their use as antiviral agents.

Background to the Invention

Phosphatidylinositol (hereinafter abbreviated as "PI") is one of a number of phospholipids found in cell membranes. In recent years it has become clear that PI plays an important role in intracellular signal transduction. In the late 1980s, a PI3 kinase (PI3K) was found to be an enzyme which phosphorylates the 3-position of the inositol ring of phosphatidylinositol (D. Whitman *et al,* 1988).

PI3K was originally considered to be a single enzyme, but it has now been clarified that a plurality of subtypes are present in PI3K. Each subtype has its own mechanism for regulating activity. Three major classes of PI3Ks have been identified on the basis of their *in vitro* substrate specificity (B. Vanhaesebroeck,1997). Substrates for class I PI3Ks are PI, PI 4-phosphate (PI4P) and PI 4,5-biphosphate (PI (4,5)P2). Class I PI3Ks are further divided into two groups, class Ia and class Ib, in terms of their activation mechanism. Class Ia PI3Ks include PI3K p110α, p110β and p110δ subtypes, which are activated in the tyrosine kinase system. Class Ib PI3K includes a p110γ subtype activated by a G protein-coupled receptor.

PI and PI(4)P are known as substrates for class II PI3Ks. Class II PI3Ks include PI3K C2α, C2β and C2γ subtypes, which are characterized by containing C2 domains at the C terminus. The substrate for class III PI3Ks is PI only.

In the PI3K subtypes, the class Ia subtype has been most extensively investigated to date. The three subtypes of class Ia are hetero dimers of a catalytic 110 kDa subunit and regulatory subunits of 85 kDa or 55 kDa. The regulatory subunits contain SH2 domains and bind to tyrosine residues phosphorylated by

growth factor receptors with a tyrosine kinase activity or oncogene products, thereby inducing the PI3K activity of the p110 catalytic subunit. Thus, the class Ia subtypes are considered to be associated with cell proliferation and carcinogenesis. Furthermore, the class Ia PI3K subtypes bind to activated ras oncogene to express their enzyme activity.

Phosphatidylinositol 3,5-biphosphate (PtdIns(3,5)P_2, or PI(3,5)P2) is thought to be involved in regulating vacuole membrane homeostasis in mammalian cells (Wurmser *et al.* 1999). The mammalian kinase p235, also known as PIKfyve, is known to generate phosphatidylinositol 3,5-bisphosphate from the PI3-phosphate (PI3P) substrate (McEwen *et al.*, 1999). Introduction of a kinase dead mutant of p235 (p235k1831E) into mammalian cells has been shown to alter vesicle morphology. Transfection of p235k1831E into cells results in the progressive accumulation of multiple swollen vacuoles of endosomal origin (Ikonomov et al., 2001) and the site action of p235 is thought to be the late endosome/multivesicular body (MVB).

In *S. cerevisae* carrying mutations in the fab1 gene encoding the yeast homologue of p235 a large poorly acidified vacuole is formed, due to the failure to synthesise PtdIns(3,5)P_2. The yeast gene vac14 is implicated in Fab1 regulation and vac14 negative cells make very little PtdIns(3,5)P_2 (Dove *et al.*, 2002). Putative vac14 orthologues include a human protein that aligns well with Vac14. A partial sequence of this protein was cloned from T-lymphocytes as TRX, a ubiquitously expressed binding partner for the multifunctional Tax1 protein of the lymphotropic retrovirus HTLV-1.

It has recently been shown that retroviruses bud from cells by appropriating cellular machinery that is normally used in vacuolar protein sorting via the MVB. Tsg101 (vps23), a member of class E proteins, has been shown to bind to a tetrapeptide motif of the structural gag protein of HIV-1. The depletion of Tsg101 arrests HIV-1 budding at a late stage in viral particle release (Demirov *et al.*, 2002; Garrus *et al.*, 2001). Recent work has also revealed that a subverted ubiquitin dependent MVB sorting machinery underlies virus budding from infected

mammalian cells: viral proteins mediate mis-targeting of the MVB machinery to the cell surface. Inhibition of ubiquitination prevents the sorting of retroviral gag proteins into forming virions and irreversible ubiquitination of these proteins overcomes this inhibition (Patnaik *et al.*, 2000; Hicke *et al.*, 2001).

Summary of the Invention

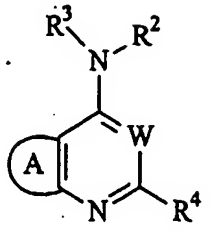
It has now been found that PtdIns(3,5)P_2 signalling is critical to the process of viral sorting, trafficking and viral budding from mammalian cells and that inhibitors of the PtdIns(3,5)P_2 pathway are effective antiviral agents *in vivo*. Accordingly, the present invention provides the use, in the manufacture of a medicament for use as an anti-viral agent, of a compound which inhibits the phosphatidylinositol 3,5-biphosphate pathway.

Detailed description of the Invention

The PI(3,5)P2 pathway comprises the steps of (i) phosphorylation of the 3-position of the PI substrate by a PI3 kinase to produce PI3P; and (ii) phosphorylation of the 5-position of the PI3P substrate by a PI3P 5-kinase to produce PI(3,5)P2. An example of a PI3P 5-kinase is the p235 enzyme.

A compound which inhibits at least one of the above steps in the pathway to PI(3,5)P2 production, or at least one substrate involved in those steps, is suitable for use in the present invention. In one aspect of the invention the compound is an inhibitor of a PI3 5-kinase, for instance an inhibitor of the p235 enzyme. In another aspect of the invention the compound is an inhibitor of a PI3 kinase. In a further aspect of the invention the compound is an inhibitor of the PI3 kinase p110α subtype enzyme. Suitable compounds for use in the invention include those disclosed in EP-A-1,277,738 (corresponding to WO 01/083456) and EP-A-1,277,754 (corresponding to WO 01/083481).

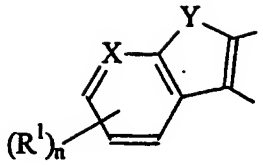
A compound according to EP-A-1,277,738 is a fused heteroaryl derivative of general formula (1)



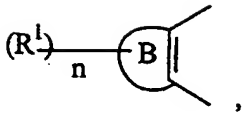
(I)

wherein:

: (a)



or (b)



,

B represents a benzene ring, or a 5- or 6-membered monocyclic heteroaryl containing 1 to 2 hetero atoms selected from O, S and N;

R^1 represents lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, aryl which may have one or more substituents, heteroaryl which may have one or more substituents, halogen, $-NO_2$, -CN, a halogenated lower alkyl, -ORb, -SRb, $-SO_2$-Rb, -SO-Rb, -COORb, -CO-Rb, -CONRaRb, $-SO_2NRaRb$, -NRaRb, -NRa-CORb, -NRa-SO_2Rb, -O-CO-NRaRb or -NRaCO-COORb, -CO-a nitrogen-containing saturated heterocyclic group, -CONRa- lower alkylene-ORb, -CONRa- lower alkylene-NRb, -O-lower alkylene-ORb, -O-lower alkylene-O-lower alkylene-ORb, -O-lower alkylene-NRaRb, -O-lower alkylene-O-lower alkylene-NRaRb, -O-lower alkylene-NRc-lower alkylene-NRaRb, -NRc- lower alkylene-NRaRb, -N(a lower alkylene-NRaRb)$_2$, -CONRa-ORb, -NRa-CO-NRbRc, or -OCORb;

each of R^2 and R^3, which may be the same or different, represents H, lower alkyl, lower alkylene-ORa or lower alkylene-NRaRc, or R^2 and R^3 are combined together with the N atom adjacent thereto to form a nitrogen-containing saturated heterocyclic group as $-NR^2R^3$ which may have one or more substituents;

each of Ra and Rc, which may be the same or different, represents H or lower alkyl;

Rb represents H, lower alkyl, cycloalkyl, aryl which may have one or more

substituents or a heteroaryl which may have one or more substituents;

n represents 0, 1, 2 or 3;

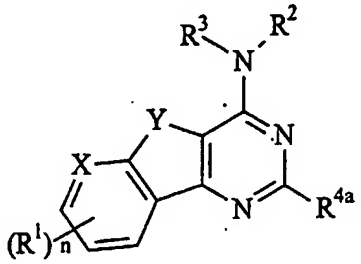
each of W and X, which may be the same or different, represents N or CH;

Y represents O, S or NH; and

R^4 represents H, lower alkyl, lower alkenyl, lower alkynyl, -(aryl which may have one or more substituents), lower alkylene-(aryl which may have one or more substituents), lower alkenylene-(aryl which may have one or more substituents), lower alkynylene-(aryl which may have one or more substituents), -(cycloalkyl which may have one or more substituents), -(cycloalkenyl which may have one or more substituents), lower alkylene-(cycloalkyl which may have one or more substituents), lower alkenylene-(cycloalkyl which may have one or more substituents), lower alkylene-(nitrogen-containing saturated heterocyclic group which may have one or more substituents), lower alkenylene-(nitrogen-containing saturated heterocyclic group which may have one or more substituents), (a heteroaryl which may have one or more substituents), lower alkylene-(heteroaryl which may have one or more substituents), or lower alkenylene-(heteroaryl which may have one or more substituents);

or a pharmaceutically acceptable salt thereof.

In one embodiment the compound is a fused heteroaryl derivative is of formula (Ia):



(Ia)

wherein R represents lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, aryl which may have one or more substituents, heteroaryl which may have one or more substituents, halogen, $-NO_2$, -CN, a halogenated lower alkyl, -ORb, -SRb, $-SO_2$-Rb, -SO-Rb, -COORb, -CO-Rb, -CONRaRb, $-SO_2NRaRb$, -NRaRb, -NRa-CORb, -NRa-SO_2Rb, O-CO-NRaRb or -NRaCO-COORb, -CO- nitrogen-containing saturated heterocyclic group, -CONRa-lower alkylene-ORb, -CONRa-lower alkylene-NRbRc, -O-lower alkylene-ORb, -O-lower alkylene-O-lower alkylene-ORb, -O-lower alkylene-NRaRb, -O-lower alkylene-O-lower alkylene-NRaRb, -O-lower alkylene-NRc-lower alkylene-NRaRb, -NRc-lower alkylene-NRaRb, -N(lower alkylene-NRaRb)$_2$, -CONRa-ORb, -NRa-CO-RbRc, or -OCORb;

each of R^2 and R^3, which may be the same or different, represents H or lower alkyl, or R^2 and R^3 are combined together with the N atom adjacent thereto to form a nitrogen-containing saturated heterocyclic group as $-NR^2.R^3$ which may have one or more substituents;

Ra and Rc, which may be the same or different, represent H or lower alkyl;

Rb represents H, lower alkyl, cycloalkyl, aryl which may have one or more substituents or heteroaryl which may have one or more substituents;

n represents 0, 1, 2 or 3;

X represents N or CH;

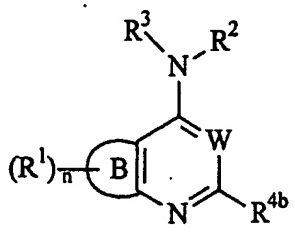
Y represents O, S or NH; and,

R^{4a} represents -(aryl which may have one or more substituents), lower alkylene-(aryl which may have one or more substituents), lower alkenylene-(aryl which may have one or more substituents), lower alkynylene-(aryl which may have one or more substituents), -(cycloalkyl which may have one or more substituents), -(cycloalkenyl which may have one or more substituents), lower alkylene-(cycloalkyl which may have one or more substituents), lower alkenylene-(cycloalkyl which may have one or more substituents), lower alkylene-(nitrogen-containing saturated heterocyclic group which may have one or more substituents), lower alkenylene-(nitrogen-containing saturated heterocyclic group which may have one or more substituents), -(heteroaryl

which may have one or more substituents), lower alkylene-(heteroaryl which may have one or more substituents), or lower alkenylene-(heteroaryl which may have one or more substituents);

or a pharmaceutically acceptable salt thereof.

In another embodiment the compound is a fused heteroaryl derivative of general formula (Ib):



(Ib)

wherein B represents a benzene ring, or a 5- or 6-membered monocyclic heteroaryl containing 1 to 2 hetero atoms selected from O, S and N;

R^1 represents lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, aryl which may have one or more substituents, heteroaryl which may have one or more substituents, halogen, $-NO_2$, -CN, halogenated lower alkyl, -ORb, -SRb, $-SO_2$-Rb, -SO-Rb, -COORb, -CO-Rb, -CONRaRb, $-SO_2$NRaRb, -NRaRb, -NRa-CORb, -NRa-SO_2Rb, -O-CO-NRaRb, -NRaCO-COORb, -NRaCOORb, -NRaCO-lower alkylene- aryl, -NRa-SO_2-lower alkylene-aryl, -NRa-lower alkylene-aryl, lower alkylene-ORb, lower alkylene-NRaRb, -CO-nitrogen-containing saturated heterocyclic group, -CONRa-lower alkylene-ORb, -CONRa-lower alkylene-NRcRb, -CONRa- lower alkylene-nitrogen-containing saturated heterocyclic group, -O-lower alkylene-ORb, -O- lower alkylene-NRaRb, -O-lower alkylene-nitrogen-containing saturated heterocyclic group, -O-lower alkylene-O-lower alkylene-ORb, -O-lower alkylene-O-lower alkylene-NRaRb, -O-lower alkylene-NRc- lower alkylene-NRaRb, -NRc-lower alkylene-NRaRb, -N(lower alkylene-NRaRb)$_2$, -CONRa-ORb, -NR-CO-

NRbRc, or -OCORb;

R^2 and R^3 are combined together with the N atom adjacent thereto to form $-NR^2R^3$ which is a nitrogen-containing saturated heterocyclic group which may have one or more substituents; Ra and Rc, which may be the same or different, represent H or lower alkyl;

Rb represents H, lower alkyl, cycloalkyl, aryl which may have one or more substituents or heteroaryl which may have one or more substituents;

n represents 0,1, 2 or 3;

W represents N or CH; and,

R^{4b} represents aryl which may have one or more substituents, lower alkylene-(aryl which may have one or more substituents), lower alkenylene-(aryl which may have one or more substituents), lower alkynylene-(aryl which may have one or more substituents), cycloalkyl which may have one or more substituents, cycloalkenyl which may have one or more substituents, lower alkylene-(cycloalkyl which may have one or more substituents), lower alkenylene-(cycloalkyl which may have one or more substituents), lower alkylene-(nitrogen-containing saturated heterocyclic group which may have one or more substituents), lower alkenylene-(nitrogen-containing saturated heterocyclic group which may have one or more substituents), -(heteroaryl which may have one or more substituents), lower alkylene-(heteroaryl which may have one or more substituents), or lower alkenylene-(heteroaryl which may have one or more substituents);

or a pharmaceutically acceptable salt thereof.

The term "lower" in formulae (I), (Ia) and (Ib) is used to mean a straight or branched hydrocarbon chain having 1 to 10, preferably 1 to 6, and more preferably 1 to 3 carbon atoms.

Typical examples of "lower alkyl" are C_1-C_6 alkyl, for instance C_1-C_4 alkyl, preferably C_1-C_3 alkyl and more preferably methyl and ethyl. Typical examples of "lower alkenyl" are C_2-C_6 alkenyl, for instance C_2-C_4 alkenyl, and include vinyl, allyl, 1-propenyl, isopropenyl, 1-butenyl, 2-butenyl and 3-butenyl. Preferred

examples of "lower alkynyl" are C_2-C_6 alkynyl, for instance C_2-C_4 alkynyl, and include ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl, 3-butynyl and 1-methyl-2-propynyl. The terms "lower alkylene", "lower alkenylene" and "lower alkynylene" are used to mean bivalent groups of lower alkyl, lower alkenyl and lower alkynyl as described above. Preferred examples of these groups are methylene, ethylene, vinylene, propenylene, ethynylene and propynylene.

The terms "cycloalkyl" and "cycloalkenyl" refer to C_3-C_8 cycloalkyl and C_3-C_8 cycloalkenyl groups, more preferably C_3-C_6 cycloalkyl and C_3-C_6 cycloalkenyl groups. Preferred examples of these groups include cyclopropyl, cyclopentyl, cyclohexyl and cyclopentenyl.

A halogen is F, Cl, Br or I. A halogenated lower alkyl group is a lower alkyl group as defined above which is substituted with one or more halogen atoms. A preferred example is CF_3.

The term "nitrogen-containing saturated heterocyclic group" in formulae (I), (Ia) and (Ib) refers to a 5- to 7-membered heterocyclic group containing one or two nitrogen atoms on the ring, which may further contain one O or S atom and may form a bridge structure or may be fused with one benzene ring. Preferred examples of such heterocyclic group are pyrrolidinyl, piperazinyl, piperidyl and morpholinyl. Preferred examples of the nitrogen-containing saturated heterocyclic group as $-NR^2R^3$ are 1-pyrrolidinyl, 1-piperazinyl, piperidino and morpholino, with particular preference to morpholino.

The term "aryl" is used in formulae (I), (Ia) and (Ib) to mean an aromatic cyclic hydrocarbon group. Typically an aryl group has 6 to 14 carbon atoms, preferably 6 to 10 carbon atoms. Suitable examples of aryl are phenyl and naphthyl.

The term "heteroaryl" in formulae (I), (Ia) and (Ib) refers to a 5- or 6-membered monocyclic heteroaryl containing 1 to 4 hetero atoms selected from N, S and O as well as a bicyclic heteroaryl fused to a benzene ring. The heteroaryl may be partially saturated. Preferred examples of the monocyclic heteroaryl are furyl, thienyl, pyrrolyl, imidazolyl, pyrazolyl, thiazolyl, isothiazolyl, oxazolyl, triazolyl, tetrazolyl,

pyridyl, pyrimidinyl, pyridazinyl and pyrazinyl. Examples of the bicyclic heteroaryl are preferably benzofuranyl, benzothienyl, benzothiadiazolyl, benzothiazolyl, benzimidazolyl, indolyl, isoindolyl, indazolyl, quinolyl, isoquinolyl, cinnolinyl, quinazolinyl, quinoxalinyl and benzodioxolyl. A specific example of the partially saturated heteroaryl is 1,2,3,4-tetrahydroquinolyl. Particularly preferred are 5- to 6-membered monocyclic groups, more preferably imidazolyl, thiazolyl, triazolyl, pyridyl and pyrazinyl.

Examples of a "5- or 6-membered monocyclic heteroaryl containing 1 or 2 hetero atoms selected from O, S and N" in B include a furan, thiophene, pyrole, imidazole, pyrazole, thiazole, isothiazole, oxazole, pyridine, pyrimidine, pyridazine and pyrazine ring. Preferably, it is a pyridine, pyrazine or thiophene ring. More preferably, it is a pyridine ring.

The substituents for the "aryl which may have one or more substituents", "heteroaryl which may have one or more substituents", "cycloalkyl which may have one or more substituents", "cycloalkenyl which may have one or more substituents" or "nitrogen-containing saturated heterocyclic group which may have one or more substituents" are from 1 to 5 substituents, which may be the same or different. Preferably, these substituents are selected from Group A as described below. Each of R, R' and R", which may be the same or different, represents H or a lower alkyl.

Group A: lower alkyl, lower alkenyl, lower alkynyl, halogen, halogenated lower alkyl, lower alkylene-OR, $-NO_2$, -CN, =O, -OR, -O-halogenated lower alkyl, -O-lower alkylene-NRR', -O-lower alkylene-OR, -O-lower alkylene-aryl, -SR, $-SO_2$-lower alkyl, -SO-lower alkyl, -COOR, -COO-lower alkylene-aryl, -COR, -CO-aryl, aryl, -CONRR', $-SO_2NRR'$, -NRR', -NR"-lower alkylene-NRR', -NR'-lower alkylene-OR, -NR-lower alkylene-aryl, -NRCO-lower alkyl, $-NRSO_2$-lower alkyl, cycloalkyl and cycloalkenyl.

When R^4, R^{4a} and R^{4b} represent "an aryl which may have one or more substituents" or "a heteroaryl which may have one or more substituents", the substituents are 1 to 5 groups selected from a) to c) below, which may be the same or

different.

a): lower alkyl, lower alkenyl, lower alkynyl, halogen, halogenated lower alkyl, lower alkylene-OR, $-NO_2$, -CN, =O, -O-halogenated lower alkyl, $-SO_2$-lower alkyl, $-SO_2$-lower alkyl, -COOR, -COO-lower alkylene- aryl, -COR, -CO-aryl, -CONRR', $-SO_2NRR'$, -Cyc or -Alp-Cyc wherein Alp represents a lower alkylene, a lower alkenylene or a lower alkynylene group, and Cyc represents an aryl which may have 1 to 5 substituents selected from Group A, a heteroaryl which may have 1 to 5 substituents selected from Group A, a nitrogen-containing saturated heterocyclic group which may have 1 to 5 substituents selected from Group A, cycloalkyl which may have 1 to 5 substituents selected from Group A or cycloalkenyl which may have 1 to 5 substituents selected from Group A.

b): -NR-E-F wherein E represents -CO-, -COO-, -CONR'-, $-SO_2NR'$- or $-SO_2$-; F represents -Cyc or lower alkyl, lower alkenyl or lower alkynyl which may be substituted by one or more substituents selected from halogen, $-NO_2$, -CN, -OR, -O-lower alkylene-NRR', -O-lower alkylene-OR, -SR, $-SO_2$-lower alkyl, -SO-lower alkyl, -COOR, -COR, -CO-aryl, -CONRR', $-SO_2NRR'$, -NRCO-lower alkyl, -NRR', -NR'-lower alkylene-OR, -NR"- lower alkylene-NRR' and -Cyc.

c): -Z-R', -Z-Cyc, -Z-Alp-Cyc, -Z-Alp-Z'-R' or -Z-Alp-Z'-Cyc wherein Cyc and Alp are as defined above and each of Z and Z', which may be the same or different, independently represents O, S or NR.

Particularly preferred are lower alkylene-OR, -CONRR', -NR-CO-Cyc1 (wherein Cyc1 is an aryl which may have from 1 to 5 substituents selected from Group A, a heteroaryl which may have from 1 to 5 substituents selected from Group A, or a nitrogen-containing saturated heterocyclic group which may have from 1 to 5 substituents selected from Group A), $-NR-SO_2-Cyc^1$, -OR, -NRR', -O-lower alkylene-NRR' and -O-lower alkylene-(nitrogen-containing saturated ring which may have from 1 to about 5 substituents selected from Group A).

When n is 2 to 4, each R^1 group is the same or different.

In the compounds of formulae (I), (Ia) and (Ib) as defined above, the

following compounds are preferred:

(1) Compounds in which R^2 and R^3 form $-NR^2R^3$ which is a nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by substituents selected from -OH, =O and lower alkyl.

(2) Compounds in which R^2 and R^3 form $-NR^2R^3$ which is morpholino.

(3) Compounds in which W is N.

(4) Compounds in which R^4, R^{4a} or R^{4b} represents an aryl which may have one or more substituents or a heteroaryl which may have one or more substituents.

(5) Compounds in which B represents a benzene ring; R^1 represents lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, aryl which may have one or more substituents, heteroaryl which may have one or more substituents, halogen, $-NO_2$, -CN, halogenated lower alkyl, -ORb, -SRb, $-SO_2$-Rb, -SO-Rb, -COORb, -CO-Rb, -CONRaRb, $-SO_2NRaRb$, -NRaRb, -NRa-CORb, $-NRa-SO_2Rb$, -O-CO-NRaRb or -NRaCO-COORb.

(6) Compounds in which B is a pyridine, pyrazine or thiophene ring and n is 0.

(7) Compounds in which X represents N, Y represents O and n is 0.

(8) Compounds in which R^4, R^{4a} or R^{4b} represents an aryl which has one or more substituents selected from a lower alkylene-OR, -CONRR', -NR-CO-Cyc^1, $-NR-SO_2$-Cyc^1, -OR, -NRR', -O-lower alkylene-NRR' and -O-lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by from 1 to 5 substituents selected from Group A).

Particularly preferred compounds of formula (Ia) are those having R^{4a} which is a phenyl having at least one substituent which is selected from -OH, $-NH_2$, -NH-lower alkyl, $-N(\text{lower alkyl})_2$, -O-lower alkylene-NH_2 and -O-lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by lower alkyl).

The following compounds of general formula (Ib) are particularly preferred:

(1) Compounds in which W represents N, R^{4b} represents aryl which is unsubstituted or substituted by one or more substituents, and R^2 and R^3 form $-NR^2R^3$

which is morpholino;

(2) Compounds in which B represents a benzene ring, n is 1 or 2, and R^1 represents halogen, $-NO_2$, -CN, halogenated lower alkyl, -ORb, -SRb, -NRaRb, -NRa-CORb or $-NRa-SO_2Rb$; and

(3) Compounds in which B represents a pyridine, pyrazine or thiophene ring, n is 0, and R^{4b} represents phenyl which has at least one substituent selected from -OH, $-CH_2OH$ and $-CONH_2$.

Specific examples of compounds of general formula (Ia) are:

6-amino-3'-(4-morpholinopyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl)nicotinanilide,

4-(4-morpholinopyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-y l)aniline,

3-(4-morpholinopyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl)phenol,

4-morpholino-2-[3-(2-piperazin-1-ylethoxy)phenyl]pyrido[3',2':4,5]furo[3,2-d]pyrimidine,

3'-(4-morpholinopyrido[3',2':4, 5]furo[3,2-d]pyrimidin-2-yl)acrylanilide,

and pharmaceutically acceptable salts thereof.

Specific examples of compounds of general formula (Ib) are:

N-[2-(3-benzenesulfonylaminophenyl)-4-morphoniloquinazolin-6-yl]acetamide-,

3-(4-morpholinopyrido[4,3-d]pyrimidin-2-yl)phenol,

3-(4-morpholinopyrido[3,2-d]pyrimidin-2-yl)phenol,

3-(4-morpholinopyrido[3,4-d]pyrimidin-2-yl)phenol,

3-(6-methoxy-4-morpholinoquinazolin-2-yl)phenol,

3-(4-morpholinothieno[3,2-d]pyrimidin-2-yl)phenol,

3-(4-morpholinopteridin-2-yl)phenol,

and pharmaceutically acceptable salts thereof.

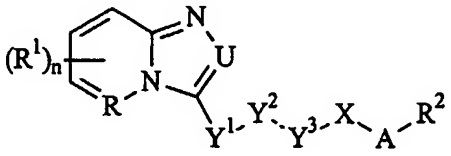
The compounds of formulae (I), (Ia) and (Ib) may exist in the form of geometrical isomers or tautomers depending on the kinds of substituent groups, and these isomers in separated forms or mixtures thereof may be used in the present invention. Where the compounds have asymmetric carbon atoms, optical isomer forms may exist based on such carbon atoms. All of the mixtures and the isolated

forms of these optical isomers may be used in the present invention.

Some of the compounds of formulae (I), (Ia) and (Ib) form salts. Specific examples of pharmaceutically acceptable salts are salts with inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, nitric acid and phosphoric acid; and organic acids such as formic acid, acetic acid, propionic acid, oxalic acid, malonic acid, succinic acid, fumaric acid, maleic acid, lactic acid, malic acid, tartaric acid, citric acid, methanesulfonic acid, ethanesulfonic acid, aspartic acid and glutamic acid. Specific examples of basic salts include salts with inorganic bases containing metals such as sodium, potassium, magnesium, calcium or aluminum, or salts with organic bases such as methylamine, ethylamine, ethanolamine, lysine, ornithine. The compounds of formula (I), (Ia) and (Ib) and their salts may exist as hydrates or solvates.

The compounds of formulae (I), (Ia) and (Ib) may be synthesized as described in EP-A-1,277,738.

A compound according to EP-A-1,277,754 is an imidazopyridine derivative of formula (II):



(II)

wherein

R^1 represents H, lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkenyl, halogen, $-NO_2$, -CN, halogenated lower alkyl, $-OR^a$, $-SR^a$, $-SO_2R^a$, $-SOR^a$, $-CO_2R^a$, $-CO-R^a$, aryl, lower alkylene-aryl, -O-lower alkylene-aryl, $-CONR^aR^b$, -CO-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), $-SO_2NR^aR^b$, $-SO_2$-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), $-SO_3H$, a nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group, $-NR^aR^b$, $-CONR^a$-lower alkylene-OR^b, $-CONR^a$-lower

alkylene-NR^bR^c, -$CONR^a$-lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), -O-lower alkylene-OR^a, -O-lower alkylene-O-lower alkylene-O^a, -O-lower alkylene-NR^aR^b, -O-lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), -O-lower alkylene-O-lower alkylene-NR^aR^b, -O-lower alkylene-O-lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), -O-lower alkylene-NR^c-lower alkylene-NR^aR^b, -O-lower alkylene-NR^c-lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), -OCO-NR^aR^b, -OCO-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), -NR^a-SO_2R^b, -NR^c-lower alkylene-NR^aR^b, -NR^c-lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), -N(lower alkylene-$NR^aR^b)_2$, -N(lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group))$_2$, -$CONR^a$-OR^b, -NR^a-COR^b, -NR^aCO-NR^bR^c, -NR^a-CO-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), or -$OCOR^a$;

R^a, R^b and R^c, which may be the same or different, are H, lower alkyl or aryl;

T is N or CR^{1a};

U is N or CR^3;

n is an integer of 1, 2 or 3;

in Y . . . Y^2. . . Y^3,

i) . . . represents a single bond on one side and a single or double bond on the other side, Y^1 represents CR^5 or $CR^{5a}R^{5b}$, Y^2 represents N, NH, CR^{4a} or $CR^{4b}R^{4c}$, and Y^3 represents NR^6, CR^{4d} or CR^{4e} R^{4f}, whereas Y^3 represents NR^6 when Y^2 represents CR^{4a} or CR^{4b} R^{4c}, or

ii) Y^1 and Y^3 may be bonded with each other via 2 or 3 atoms and combined with the adjacent Y^2 to form a B ring, wherein the B ring represents a 5- or 6-membered

monocyclic heteroaryl ring having 1 to 4 hetero atoms selected from N, S, and O, a nitrogen-containing saturated heterocyclic ring or an aryl ring, whereas said B ring may be substituted by one to two R^4s;

X represents S, SO or SO_2, and X may also represent CO, NR^7 or a methylene group when Y^1 and Y^3 are bonded with each other via 2 or 3 atoms and combined with the adjacent Y^2 to form the B ring;

A represents a direct linkage, lower alkylene, lower alkenylene or lower alkenylene;

R^2 represents lower alkyl which is unsubstituted or substituted by one or more subsituents, lower alkenyl which is unsubstituted or substituted by one or more subsituents, a lower alkynyl which is unsubstituted or substituted by one or more subsituents, cycloalkyl which is unsubstituted or substituted by one or more subsituents, a cycloalkenyl which is unsubstituted or substituted by one or more subsituents, -N=O, aryl which is unsubstituted or substituted by one or more subsituents, or heteroaryl which is unsubstituted or substituted by one or more subsituents;

R^{1a}, R^3, R^{4a}, R^{4c}, R^{4d}, R^{4e}, R^{4f}, R^{5a} and R^{5b}, which may be the same or different, represent a group defined by R^1, or R^{4b} and R^{4c}, R^{4e} and R^{4f}, or R^{5a} and R^{5b} may be combined with each other to form an oxo group (=O);

R^4 represents a group defined by R^1, or an oxo group (=O);

R^5, R^6 and R^7, which may be the same or different, represent -H, a lower alkyl which is unsubstituted or substituted by one or more subsituents, lower alkenyl which is unsubstituted or substituted by one or more subsituents, or lower alkynyl which is unsubstituted or substituted by one or more substituents;

or a pharmaceutically acceptable salt thereof.

In formula (II) preferred examples of "lower alkyl" are C_1-C_6, for instance C_1-C_4 alkyl, more preferably methyl and ethyl. The term "lower alkenyl" includes C_2-C_6 alkenyl, for instance C_2-C_4 alkenyl and is, for instance vinyl, allyl, 1-propenyl, isopropenyl, 1-butenyl, 2-butenyl or 3-butenyl.. A "lower alkynyl" is C_2-C_6 alkynyl, for instance C_2-C_4 alkynyl, and includes ethynyl, 1-propynyl, 2-propynyl, 1-butynyl,

2-butynyl, 3-butynyl and 1-methyl-2-propynyl. The terms "cycloalkyl" and "cycloalkenyl" are cycloalkyl and cycloalkenyl, having 3 to 8 carbon atoms, typically 3 to 6 carbon atoms, and are preferably cyclopropyl, cyclopentyl, cyclohexyl and cyclopentenyl. Preferred examples of the "lower alkylene" include methylene, ethylene, trimethylene and 2,2-dimethyltrimethylene. The "lower alkenylene" is preferably vinylene. The "lower alkenylene" is preferably ethynylene.

In formula (II) the term "aryl" is used to mean an aromatic cyclic hydrocarbon group. An aryl having 6 to 14 carbon atoms typically 6 to 10 carbon atoms, is preferable. It may be partially saturated. Preferred examples of such aryl are phenyl and naphthyl. When Y^1 and Y^3 are bonded via 2 or 3 atoms and combined with the adjacent Y^2 to form a B ring, a preferred example of the B ring is a benzene or naphthalene ring.

In formula (II) the term "heteroaryl" includes a 5- or 6-membered monocyclic heteroaryl having 1 to 4 hetero atoms selected from the group consisting of N, S and O as well as a bicyclic heteroaryl in which such a monocyclic heteroaryl is fused to a benzene ring. The heteroaryl may be partially saturated. A 5- to 6-membered monocyclic heteroaryl having 1 to 4 hetero atoms selected from the group consisting of N, S, and O is preferably exemplified by furyl, thienyl, pyrolyl, imidazolyl, pyrazolyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl, triazolyl, tetrazolyl, thiadiazolyl, pyridyl, pyrimidinyl, pyridazinyl, and pyrazinyl. Examples of the bicyclic heteroaryl are preferably benzofuranyl, benzothienyl, benzothiadiazolyl, benzothiazolyl, benzimidazolyl, indolyl, isoindolyl, indazolyl, quinolyl, isoquinolyl, cinnolinyl, quinazolinyl, quinoxalinyl and benzodioxolyl. Specific examples of the partially saturated heteroaryl are 1,2,3,4-tetrahydroquinolyl, etc. Particularly preferred as a heteroaryl in R^2 are thienyl, pyrazolyl, thiazolyl, isoxazolyl, pyridyl, benzothiadiazolyl and quinolyl.

In formula (II) a "5- to 6-membered monocyclic heteroaryl ring having 1 to 4 hetero atoms selected from the group consisting of N, S, and O" in a B ring formed by bonding Y^1 with Y^3 via 2 or 3 atoms and combining Y^1 and Y^3 with the adjacent

Y^2 is a heteroaryl ring forming the aforementioned "5- to 6-membered monocyclic heteroaryl having 1 to 4 hetero atoms selected from the group consisting of N, S, and O". Preferable examples are a furan, thiophene, pyrrole, imidazole, pyrazole, thiazole, isothiazole, oxazole, isoxazole, triazole, tetrazole, thiadiazole, pyridine, pyrimidine, pyridazine and pyrazine ring. More preferable examples are a 5-membered monocyclic heteroaryl ring. Among them, more preferable examples are a pyrrole, imidazole, pyrazole, thiazole, oxazole and triazole ring, and particularly preferable examples are a pyrazole and thiazole ring.

Examples of "halogen" and "halogenated lower alkyl" in formula (II) are as defined above for formulae (I), (Ia) and (Ib).

In formula (II) the "nitrogen-containing saturated heterocyclic group" is a 5- to 7-membered heterocyclic group containing one or two nitrogen atoms on the ring, which may further contain one O or S atom and may form a bridge structure. Preferred examples of such heterocyclic group are 1-pyrrolidinyl, 1-piperazinyl, piperidino and morpholino. The "5- to 7-membered nitrogen-containing heterocyclic ring fused with a benzene ring(s) of the aryl group" which is formed by combining R^7 "with the substituent at the ortho-position to form a C_2-C_3 lower alkylene chain, and with the aryl of R^2 "when X is NR^7 and R^2 is an aryl having a substituent at the ortho-position" includes the above defined "nitrogen-containing saturated heterocyclic group" fused with an aryl ring(s), preferably 1-pyrrolidinyl and piperidino fused with a benzene ring. The "nitrogen-containing saturated heterocyclic group" in the B ring formed by bonding Y^1 with Y^3 via 2 or 3 atoms and combining Y^1 and Y^3 with the adjacent Y^2 is preferably a pyrrolidine, imidazolidine or pyrazolidine ring and more preferably is a pyrrolidine ring.

In formula (II) the subsituents in the "lower alkyl which is unsubstituted or substituted", "lower alkenyl which is unsubstituted or substituted" and "lower alkynyl which is unsubstituted or substituted" are 1 to 5 subsituents selected from Group D below.

Group D comprises a halogen, $-NO_2$, -CN, -OH, -O-lower alkyl, -O-

halogenated lower alkyl, -SH, -S-lower alkyl, -SO_2-lower alkyl, -SO-lower alkyl, -COOH, -COO-lower alkyl, -CO-lower alkyl, -$CONH_2$, -NH_2, -NH-lower alkyl, -N(lower alkyl)$_2$, nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group, aryl, heteroaryl, cycloalkyl and cycloalkenyl.

In formula (II) the substituent(s) for "cycloalkyl which is unsubstituted or substituted by one or more subsituents", " cycloalkenyl which is unsubstituted or substituted by one or more subsituents", " aryl which is unsubstituted or substituted by one or more subsituents", and " heteroaryl which is unsubstituted or substituted by one or more subsituents" shown by R^2 are preferably lower alkyl which is unsubstituted or substituted by 1 to 5 substituents which are selected from Group D, a lower alkenyl which is unsubstituted or substituted by 1 to 5 substituents which are selected from Group D, a lower alkynyl which is unsubstituted or substituted by 1 to 5 substituents which are selected from Group D, a cycloalkyl which is unsubstituted or substituted by 1 to 5 substituents which are selected from Group E as defined below, cycloalkenyl which is unsubstituted or substituted by 1 to 5 substituents which are selected from Group E, halogen, -NO_2, -CN, halogenated lower alkyl, -O-halogenated lower alkyl, -OH, -O-lower alkyl, -SH, -S-lower alkyl, -SO_2-lower alkyl, -SO-lower alkyl, -COOH, -COO-lower alkyl, -CHO, -CO-lower alkyl, -SO_3H, -Ar^1, -O-Ar^1, -S-Ar^1, -CO-Ar^1, -SO_2-Ar^1, -SO-Ar^1, -lower alkylene-Ar^1, -O-lower alkylene-Ar^1, -$CONH_2$, -CONH-lower alkyl, -CON(lower alkyl)$_2$, -SO_2NH_2, -SO_2NH-lower alkyl, -SO_2N(lower alkyl)$_2$, -CO-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), -SO_2-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group, -NH_2, -NH-lower alkyl, -N(lower alkyl)$_2$, -NHCO-lower alkyl, -NHCO-Ar^1, -$NHSO_2$-lower alkyl, -$NHSO_2$-Ar^1, -azido and -N=N-Ar^1, wherein Group E comprises lower alkyl, lower alkenyl, lower alkynyl and the substituents in Group D as defined above, and wherein Ar^1 is an aryl or a

heteroaryl which may have 1 to 5 substituents selected from Group E.

When n in formula (II) is 2 or 3, the R^1 groups may be the same or different. When two R^4 groups exist, each R^4 group may be the same or different from each other.

R^3, R^{4a}, R^{4b}, R^{4c}, R^{4d}, R^{4e}, and R^{4f} are preferably H, -OH, or lower alkyl. Alternatively, R^{4b} and R^{4c} may be combined with each other to form an oxo group (=O). For R^4, lower alkyl, =O, -COOH, -COO-lower alkyl, -CO-lower alkyl or -SO_3H is preferred. For R^5 and R^7, H or lower alkyl is preferred. As for R^6, H, lower alkyl or alkenyl is preferred, wherein the lower alkyl or alkenyl group is unsubstituted or substituted by a substituent(s) selected from -O-lower alkyl, -S-lower alkyl, -SO_2 -lower alkyl, -SO-lower alkyl, -COOH, -COO-lower alkyl, -CO-lower alkyl, -$CONH_2$, -NH_2, -NH-lower alkyl, -N(lower alkyl)$_2$, nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by lower alkyl, and aryl.

Preferred imidazopyridine derivatives of formula (II) are as follows:

(1) Compounds in which R^1 is H, lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkenyl, halogen, -NO_2, -CN, halogenated lower alkyl, -OH, -O-lower alkyl, -O-aryl, -SH, -S-lower alkyl, -SO_2-lower alkyl, -SO-lower alkyl, -COOH, -COO-lower alkyl, -CO-lower alkyl, -aryl, -CO-aryl, -lower alkylene-aryl, -O-lower alkylene-aryl, -$CONH_2$, -SO_2NH_2, -SO_3H, a nitrogen-containing saturated heterocyclic group, -NH_2, -NH-lower alkyl or -N(lower alkyl)$_2$; T is CR^{1a}; U is CR^3; in $Y^1 \ldots Y^2 \ldots Y^3$, i) . . . represents a single bond on one side and a single or double bond on the other side, Y^1 represents CR^5 or CHR^{5a}, Y^2 represents N, CR^{4a} or CHR^{4b}, and Y^3 represents NR^7, CR^{4d} or CHR^{4e}, or ii) Y^1 and Y^3 may be bonded with each other via 2 or 3 atoms and combined with the adjacent Y^2 to form a B ring, wherein the B ring represents a 5- or 6-membered monocyclic heteroaryl ring having 1 to 4 hetero atoms selected from the group consisting of N, S, and O, or an aryl ring, whereas the B ring may be substituted by one to two R^4s; R^2 represents H, halogenated lower alkyl, -N=O, aryl which is unsubstituted or substituted by one or

more subsituents, or heteroaryl which is unsubstituted or unsubstituted by one or more subsituents; R^{1a}, R^3, R^4, R^{4a}, R^{4b}, R^{4d} and R^{4e}, which may be the same or different, represent a group defined by R^1; and R^5, R^{5a}, R^6 and R^7, which may be the same or different, represent H or lower alkyl, with proviso that when X is NR^7 and R^2 is an aryl having a substituent at the ortho-position, R^7 may be combined with the substituent at the ortho-position to form a C_{2-3} lower alkylene chain, and with the aryl of R^2 to form a 5- to 7-membered nitrogen-containing heterocyclic ring fused with a benzene ring(s) of the aryl group.

(2) Compounds in which n is 1 and R^1 represents lower alkyl, halogen, -CN, -NO_2, halogenated lower alkyl, -OR^a, -O-lower alkylene-aryl, -CO_2R^a, -$CONR^a$ - lower alkylene-OR^b, -$CONR^aR^b$, -CO-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by lower alkyl), -$CONR^a$-lower alkylene-NR^bR^c, -$CONR^a$-lower alkylene-(nitrogen-containing saturated heterocyclic group which may be substituted by a lower alkyl group) or aryl.

(3) Compounds in which "A" represents a linkage and R^2 represents an aryl which is unsubstituted or substituted by one or more subsituents or a heteroaryl which is unsubstituted or substituted by one or more subsituents.

(4) Compounds in which R^2 represents a phenyl which is unsubstituted or substituted by one or more substituents which are selected from (lower alkyl which is unsubstituted or substituted by -OH), lower alkenyl, halogen, -NO_2, -CN, halogenated lower alkyl, -O-halogenated lower alkyl, -OH, -O-lower alkyl, -CO-lower alkyl, -SO_2-lower alkyl, -COOH, -COO-lower alkyl, -$CONH_2$, -SO_2NH_2, -CO-aryl, -SO_2-aryl, -NH_2, -NH-lower alkyl, -N(lower alkyl)$_2$, -(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), -NHCO-lower alkyl, aryl which is unsubstituted or substituted 1 to 5 substituents selected from Group E, and heteroaryl which is unsubstituted or substitued by 1 to 5 substituents selected from Group E.

(5) Compounds in which T represents CH and U represents CH or C-(lower alkyl).

(6) Compounds in which X represents SO_2.

(7) Compounds in which i) $Y^1 \ldots Y^2 \ldots Y^3$ represents $CR^5=N-NR^6$, $CR^{5a}R^{5b}-NH-NR^6$, $CR^{5a}R^{5b}-CR^{4b}R^{4c}-NR^6$, or ii) Y^1 and Y^3 of $Y^1 \ldots Y^2 \ldots Y^3$ are bonded with each other via 2 or 3 atoms and combined with the adjacent Y^2 to form a 5- or 6-membered monocyclic heteroaryl ring, wherein said 5- or 6-membered monocyclic heteroaryl ring may be substituted by one to two R^4s.

(8) Compounds in which a chain structure or a partial structure of a monocyclic heteroaryl ring in the group $Y^1 \ldots Y^2 \ldots Y^3$, contains a frame which is represented by "C=N-N", "C=N-C" or "C-N=C", preferably "C=N-N".

(9) Compounds in which $Y^1 \ldots Y^2 \ldots Y^3$ represents $CR^5=N-NR^6$, R^5 represents H or lower alkyl, and R^6 represents H, or lower alkyl or alkenyl which may is unsubstituted or substituted by one or more substituents selected from -O-lower alkyl, -S-lower alkyl, -SO_2 lower alkyl, -SO-lower alkyl, -COOH, -COO-lower alkyl, -CO-lower alkyl, -$CONH_2$, -NH_2, -NH-lower alkyl, -N(lower alkyl)$_2$, -(nitrogen-containing saturated heterocyclic group which is unsubstituted to substituted by a lower alkyl group), and aryl.

(10) Compounds in which Y^1 and Y^3 of $Y^1 \ldots Y^2 \ldots Y^3$ are bonded with each other via 2 or 3 atoms and combined with the adjacent Y^2 to form a 5-membered monocyclic heteroaryl ring which may be substituted by one to two R^4s selected from lower alkyl, -COOH, -COO-lower alkyl, -CO-lower alkyl and -SO_3H.

Specific examples of compounds of formula (II) are:

3-(6-Bromo-2-methylimidazo[1,2-a]pyridin-3-yl)-1H-pyrazol-1-yl 2-methyl-5-nitrophenyl sulfone;

3-(6-bromoimidazo[1,2-a]pyridin-3-yl)-1H-pyrazol-1-yl 2-methyl-5-nitrophenyl sulfone;

2'-[(6-bromoimidazo[1,2-a]pyridin-3-yl)methylidene]-1',2-dimethyl-5-nitrobenzenesulfonohydrazide;

2'-[(6-bromoimidazo[1,2-a]pyridin-3-yl)methylidene]-2-ethyl-1'-methyl-5-nitrobenzenesulfonohydrazide;

3-({2-[(6-bromoimidazo[1,2-a]pyridin-3-yl)methylidene]-1-methylhydrazino}sulfonyl)4-methylbenzonitrile;

2'-[(6-fluoroimidazo[1,2-a]pyridin-3-yl)methylidene]-1',2-dimethyl-5-nitro benzenesulfonohydrazide;

2-amino-2'-[(6-chloroimidazo[1,2-a]pyridin-3-yl)methylidene]-1'-methyl-5-nitrobenzenesulfonohydrazide;

2'-[(6-chloroimidazo[1,2-a]pyridin-3-yl)methylidene]-1'-methyl-5-nitro-2-(2,2,2-trifluoroethoxy)benzenesulfonohydrazide;

6-chloro-3-[2-(2-methyl-5-nitrobenzenesulfonyl)thiazol-4-yl]imidazo[1,2-a] pyridine;

6-bromo-3-{[(2-methyl-5-nitrobenzenesulfonyl)(2-morpholinoethyl)hydrazono] methyl}imidazo[1,2-a]pyridine;

6-chloro-3-{[(methyl)(2-methyl-5-nitrobenzenesulfonyl)hydrazono]methyl}imidazo[1,2-a]pyridine;

3-{[(methyl)(2-methyl-5-nitrobenzenesulfonyl)hydrazono]methyl}imidazo[1,2-a]pyridine-6-carbonitrile;

5-cyano-2'-[(6-fluoroimidazo[1,2-a]pyridin-3-yl)methylidene]-1',2-dimethyl benzenesulfonohydrazide;

5-cyano-2'-[(6-cyanoimidazo[1,2-a]pyridin-3-yl)methylidene]-1',2-dimethylbenzenesulfonohydrazide;

1'2-dimethyl-2'-[(6-methylimidazo[1,2-a]pyridin-3-yl)methylidene]-5-nitrobenzenesulfonohydrazide;

2'-[(6-chloroimidazo[1,2-a]pyridin-3-yl)methylidene]-2-(1H-Imidazol-1-yl)- 1'-methyl-5-nitrobenzenesulfonohydrazide;

2'-[(6-chloroimidazo[1,2-a]pyridin-3-yl)methylidene]-2- dimethylamino-1'-methyl-5-nitrobenzenesulfonohydrazide;

and pharmaceutically acceptable salts thereof.

The compounds of formula (II) may be synthesized as described in EP-A-1,277,754.

A compound for use in the present invention may be identified as an inhibitor of the PtdIns(3,5)P_2 pathway by any suitable assay, for example a kinase assay. PI3 kinases are well known and assays for identifying inhibitors of PI3K are documented in the art. For example, EP-A-1,277,754 and EP-A-1,277,738 describe a PI3K (p110α subtype) inhibition assay. PI3P 5-kinases are also known and assays for identifying inhibitors of PI3P 5-kinases are described in the literature. A typical procedure is described in the Examples which follow.

A compound which is suitable for use in the present invention may be a selective inhibitor for one step in the PtdIns(3,5)P_2 pathway. For instance, it may selectively inhibit the PI3P 5-kinase over the PI3 kinase, or vice versa. Such selectivity is typically reflected in the relative IC50 values. The IC50 value in inhibiting the PI3P 5-kinase, such as the p235 enzyme, may be lower than the IC50 value in inhibiting the PI3 kinase, such as the PI3K p110α enzyme, reflecting greater potency against the PI3P 5-kinase.

A compound for use in the present invention typically inhibits at least one step in the PtdIns(3,5)P_2 pathway with an IC50 value of less than 100 μM, typically less than 10 μM, preferably less than 1 μM and more preferably less than 0.1 μM.

A compound which is an inhibitor of the PtdIns(3,5)P_2 pathway is useful as an anti-viral agent, as described above and as illustrated in the Examples which follow. The compound may be used to prevent or treat an infection caused by a virus. Accordingly a human or animal harbouring, or at risk of contracting, a viral infection may be treated by a method which comprises the administration thereto of a compound which inhibits the PtdIns(3,5)P_2 pathway.

The virus causing the infection may be a budding virus. In particular it may be a retrovirus. There are three known categories of retrovirus: Oncovirinae cause

sarcomas and leukemia and include Rous Sarcoma Virus; Lentivirinae cause slow progressive degenerative disorders and include HIV; and Spumavirinae represent the third category and have unknown effects. Specific examples of retroviruses include human T-cell leukemia virus Type 1 (HTLV-1) and Type 2 (HTLV-2), and Human Immunodeficiency Virus (HIV).

A compound for use in the present invention can be administered in a variety of dosage forms, for example orally such as in the form of tablets, capsules, sugar- or film-coated tablets, liquid solutions or suspensions or parenterally, for example intramuscularly, intravenously or subcutaneously. The compound may therefore be given by injection or infusion.

The dosage depends on a variety of factors including the age, weight and condition of the patient and the route of administration. Typically, however, the dosage adopted for each route of administration when a compound is administered alone to adult humans is 0.0001 to 50 mg/kg, most commonly in the range of 0.001 to 10 mg/kg, body weight, for instance 0.01 to 1 mg/kg. Such a dosage may be given, for example, from 1 to 5 times daily. For intravenous injection a suitable daily dose is from 0.0001 to 1 mg/kg body weight, preferably from 0.0001 to 0.1 mg/kg body weight.

A compound which inhibits the PtdIns(3,5)P_2 pathway is formulated for use as a pharmaceutical or veterinary composition also comprising a pharmaceutically or veterinarily acceptable carrier or diluent. The compositions are typically prepared following conventional methods and are administered in a pharmaceutically or veterinarily suitable form. An anti-viral agent comprising compound which inhibits the PtdIns(3,5)P_2 pathway is therefore provided.

The compound may be administered in any conventional form, for instance as follows:

A) Orally, for example, as tablets, coated tablets, dragees, troches, lozenges, aqueous or oily suspensions, liquid solutions, dispersible powders or granules,

emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known in the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations.

Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, dextrose, saccharose, cellulose, corn starch, potato starch, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, maize starch, alginic acid, alginates or sodium starch glycolate; binding agents, for example starch, gelatin or acacia; lubricating agents, for example silica, magnesium or calcium stearate, stearic acid or talc; effervescing mixtures; dyestuffs, sweeteners, wetting agents such as lecithin, polysorbates or lauryl sulphate. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and adsorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. Such preparations may be manufactured in a known manner, for example by means of mixing, granulating, tableting, sugar coating or film coating processes.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is present as such, or mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example, sodium carboxymethylcellulose, methylcellulose,

hydroxypropylmethyl-cellulose, sodium alginate, polyvinylpyrrolidone gum tragacanth and gum acacia; dispersing or wetting agents may be naturally-occurring phosphatides, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides for example polyoxyethylene sorbitan monooleate.

The said aqueous suspensions may also contain one or more preservatives, for example, ethyl or n-propyl p-hydroxybenzoate, one or more colouring agents, such as sucrose or saccharin.

Oily suspension may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol.

Sweetening agents, such as those set forth above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by this addition of an antioxidant such as ascorbic acid. Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavouring and colouring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oils, or a mineral oil, for example liquid paraffin or mixtures of these.

Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally occuring phosphatides, for example soy bean lecithin, and esters or partial esters derived from fatty acids an hexitol anhydrides, for example sorbitan mono-oleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavouring agents. Syrups and elixirs may be formulated with sweetening agents, for example glycerol, sorbitol or sucrose. In particular a syrup for diabetic patients can contain as carriers only products, for example sorbitol, which do not metabolise to glucose or which only metabolise a very small amount to glucose.

Such formulations may also contain a demulcent, a preservative and flavouring and coloring agents;

B) Parenterally, either subcutaneously, or intravenously, or intramuscularly, or intrasternally, or by infusion techniques, in the form of sterile injectable aqueous or oleaginous suspensions. This suspension may be formulated according to the known art using those suitable dispersing of wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic paternally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol.

Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition fatty acids such as oleic acid find use in the preparation of injectables;

C) By inhalation, in the form of aerosols or solutions for nebulizers;

D) Rectally, in the form of suppositories prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperature but liquid at

the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and poly-ethylene glycols;

E) Topically, in the form of creams, ointments, jellies, collyriums, solutions or suspensions.

Brief Description of the Drawings

Figure 1 shows the specific inhibition of *in vivo* PtdIns(3,5)P_2 production by compound (1).

Figure 2 shows that compound (1) does not block serum stimulation of PKB phosphorylation.

(A) NIH3T3 cells serum starved for 18hrs (0.1% DCS) were pre-treated for 15 min with vehicle (-), 800nM YM201636, 20nM rapamycin, or 10μM LY294002. The cells were then left unstimulated or stimulated for 15 min with 10% DCS. The blots were probed with anti-PKB-P_{473} (PW88).

(B) NIH3T3 cells serum starved for 18hrs (0.1% DCS) were pre-treated for 15 min with vehicle (-), 10μM LY294002 or increasing amounts of compound (2). The cells were then left unstimulated or stimulated for 15 min with 10% DCS. The top panel shows blots probed with anti-PKB-P_{473} (Signal Tranduction Laboratories). The bottom panel shows blots probed with anti-PKB (Signal Tranduction Laboratories) to show equal loading and expression.

Figure 3 shows that siRNA knock out of p235/PIKfyve induces a swollen vesicle phenotype. Ectopic expression of yeast Fab1p rescues the compound (1) swollen vesicle phenotype.

(A) Phase constrast images from Zeiss low light microscope (magnification x 63) of NIH3T3 cells transfected with siRNA to PIKfyve/p235. Inserts show close up views illustrating the diversity of the swollen vesicles and their intra-lumenal content.

(B) pEGFPC1 empty vector or containing yeast *fab1* were ectopically

expressed in NIH3T3 cells on coverslips. On day 2 after transfection the cells were treated for 2 hours with 800nM of compound (1). The cells were then fixed and mounted. Under phase contrast at magnification x63, random fields were chosen and the number of vesicles (>1.0μM) per cell was calculated. The data represent three independent transfections counting vesicles in a total of 63 cells for pEGFPC1 and a total of 85 cells for the *fab1* cells.

Figure 4 shows that compound (1) blocks retroviral release.

(A) NIH3T3 colonies derived from puromycin selected cells infected with retrovirus containing pBabe-puro produced by the packaging line TEGH human fibrosarcoma (TEFLY Mo) treated with 0nM, 400nM or 800nM compound (1). The number of colonies is averaged from three independent experiments performed in duplicate and plotted as a percentage of the control compound (1) untreated samples. The colonies resulting from the infections using virus dilutions of 1/100 and 1/1000 were used to calculate the numbers.

(B) A representative selection of NIH3T3 cell plates of puromycin selected cells stained with crystal violet, which had been infected with a 1/1000th dilution of virus containing media from retro-viral packaging cells treated with the indicated concentrations of compound (1).

(C) Thin section Electron Micrographs of the retro-virus packaging line TEGH human fibrosarcoma (TEFLY Mo) untreated (top panel) or treated with 800nM compound (1) for 12 hours (bottom pane).

The invention will be further described in the Examples which follow with reference to the accompanying Figures.

Example 1: Experimental Procedures

Plasmid constructs

The plasmid constructs used have been described previously (Dhand *et al.* 1994 and McEwen *et al.* 1999).

In vitro lipid kinase assays

Gst-p235 was expressed in yeast, purified and assayed for kinase activity in the presence and absence of the following three compounds.

(1) 6-amino-3-(4-morpholinopyrido[3',2':4,5]furo [3,2-d] pyrimidin-2-yl)nicotinanilide

(2) 3-(4-morpholinopyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl) phenol

(3) 2'-[(6-bromoimidazo[1,2-a]pyridin-3-yl)methylidene]-1',2-dimethyl-5-nitrobenzenesulfonohydrazide.

These compounds were used at a range of concentrations to determine approximate IC50 values. Products were separated on TLC and cpm in Pi3,5P_2 spots was determined by autoradiography.

10 µl aliquots of GST-p235 bound to glutathione agarose were washed in 1 ml of kinase buffer (25 mM HEPES pH 7.4, 120mM NaCl, 5mM 2-glycerol phosphate, 0.2mM EDTA, 1 mM DTT). $MgCl_2$ solution (10µl 9.7mM) in kinase buffer was added to the enzyme and the aliquots were stored on ice until use. Lipid vesicles containing phosphatidylinositol (PtdIns) in a background of phosphatidylethanolamine (PtdEth) (in a ratio of 1:4) were prepared by drying appropriate amounts of lipid down in vacuo and bath sonicating into kinase buffer. Kinase assays were started by the addition of 55µl of kinase buffer containing 10µCi [32P]-gamma-ATP, 59µM ATP, 118µM phosphatidylinsitol 3-phosphate (PtdIns3P) (Cell Signals), 472µM PtdEth and appropriate amounts of inhibitor and transfer of the assay to a water bath at 30°C. Reactions were terminated by the addition of 237µl

of a 2:1 solution of MeOH:$CHCl_3$. The lipid productions were extracted and analysed by TLC. Values for IC50 were determined using Prism. Gst-Fab1 and Gst-p110α kinase activity in the presence and absence of test compounds were determined in the same manner.

In vivo phospholipid labelling

NIH3T3 cells were metabolically labelled with [^{32}P] orthophosphate for 1 hour in phosphate free medium, serum deprived and then serum stimulated in the presence or absence of compound (1) for 20 minutes. The cells were lysed and lipids were extracted and subjected to TLC to resolve the lipids which were then deacylated and separated by HPLC.

Western Blots

NIH3T3 cells were seeded on to 6-well plates (200,000 per well) and grown in Dulbecco's modified Eagle's Medium (DMEM) with 10% donor calf serum (DCS). The cells were then washed with DMEM and placed in DMEM with 0.1% DCS for 18 hours. The cells were pre-treated for 15 minutes (min) with vehicle dimethyl sulphoxide, DMSO), 800nM compound (1), 20nM rapamycin (10mM stock dissolved in ethanol, from Sigma), or 10μM LY294002 (Calbiochem). The cells were then either left unstimulated or were stimulated for a further 15 min with 10% DCS. Following these treatments the cells were placed on ice, washed immediately with Phosphate Buffered Saline (4°C) and harvested in 250μl 1X sample buffer. The extracts were resolved by SDS-PAGE on a 10% polyacrylamide gel, transferred onto PVDF membrane by semi-dry blotting and detecting using specific antibodies, which were visualised by ECL (Amersham). The following primary antibodies were used: PW88 a rabbit phospho-specific polyclonal antibody raised against P-ser473 of PKB (1:1000); PW56 a rabbit polyclonal anti-PKB (used at 1:1000); a rabbit polyclonal anti-P-ser473 from Signal Transduction laboratories (used at 1:1000).

Small Interfering RNA (siRNA)

Small interfering RNA were designed and synthesised using the "Silencer siRNA construction kit" (Ambion). Four regions were selected based on AA sequence, low GC content and unique sequence. NIH3T3 cells and Cos7 cells were seeded on acid washed glass coverslips in 6-well plates. They were then transfected with approximately 2µg siRNA using Lipofectamine transfection reagent (Invitrogen). The cells were monitored over 48hrs (distinct changes were observed after 24hrs) then the cells were washed and fixed in 4% paraformaldehyde for 15min. The cells were mounted on glass slides under MOWIOL (100mM TrisHCl pH 8.8, 10% (w/v) MOWIOL 4-88 (Calbiochem) and 25% (v/v) glycerol) examined using a confocal laser scanning microscope (Axioplan2 with LSM 510 Carl Zeiss Inc.) equipped with 63x/1.4 Plan-APOCHROMAT Ph3 oil immersion objective under phase-contrast.

Protein Degradation Assay

The protein degradation assay was based on the original method described by Gonostajski & Pardee (1984).

NIH3T3 cells were labelled with C^{14}-leucine (0.2µCi/ml) in DMEM (without leucine) supplemented with 10% dialysed donor calf serum (DCS) and 2mM cold leucine for 24 hours. This was followed by a chase in DMEM (without leucine) supplemented with 10% dialysed DCS and 2mM cold leucine for 24 hours. The cells were washed and then placed in either (i) DMEM (without leucine) supplemented with 10% dialysed DCS, 2mM cold leucine and 0nM compound (1), (ii) DMEM (without leucine) supplemented with 10% dialysed DCS, 2mM cold leucine and 800nM compound (1), (iii) Earl's balanced salts, 0.1% BSA (Sigma) 2mM cold leucine and 0nM compound (iv) Earl's balanced salts, 0.1% BSA (Sigma) 2mM cold leucine and 800nM compound (1) 200µl samples of medium were taken at each time point indicated. TCA to 5% was added to each sample and the cpm in the TCA

soluble material was measure. After the final sample was taken the cells were harvested and the cpm in the total acid insoluble bulk protein was determined. % protein degradation = acid soluble cpm in medium / Acid insoluble cpm in bulk protein + Acid soluble cpm in medium.

Viral Production Assays

The TEGH human fibrosarcoma line (TEFLY Mo) was used to produce ecotropic retrovirus containing pBabe-puro. Virus titre was measured by infecting NIH3T3 with dilutions of the virus containing medium and selection of the NIH3T3 cells in the presence of puromycin. Cells were washed, treated with compound or vehicle for the duration of the experiment. Culture fluid harvested and used to infect a reporter line selecting for puromycin resistance. Colonies were stained with crystal violet and counted.

Example 2: Compound (1) is a selective inhibitor of p235

A project designed to identify phosphatidylinositide 3-kinases inhibitors yielded a number of inhibitors of this class of lipid kinases. One of these compounds, compound (1), was found to have potent *in vitro* inhibitory activity against p235, with an IC50 value of 33nM (Table 1).

Table 1: *In Vitro* inhibitory properties of compounds (1) to (3)

Inhibitor	p235 IC50	Fab1 IC50	p110α IC50
compound (1)	0.033μM	>10μM	0.755μM
compound (2)	0.069μM	>10μM	0.004μM
compound (3)	3.4μM	>10μM	0.0003μM

Notably, the yeast homologue of p235, Fab1p, was found to be insensitive to compound (1) up to10μM; in addition Fab1 was found to be insensitive to LY294002.

For comparison the PI 3-kinase p110α was also assessed *in vitro* and under the same assay conditions as employed for p235 (50μM ATP), an IC50 for PI 3-Kinase was estimated at 755nM, i.e. more than 20- fold higher than for p235 (Table 1). Compound (2), which is structurally related to compound (1), displayed a significant increase in activity towards p110α with an IC50 of 4nM, whilst showing a decreased ability to inhibit p235 (Table 1). Compound (3), which is structurally unrelated to compounds (1) and (2), clearly illustrates the fact that distinctive inhibitory properties can be developed for these related lipid kinases.

Example 3: In vivo inhibition of $PI3,5P_2$ production by compound (1)

To test the *in vivo* effects of compound (1) on phosphoinositide production, NIH3T3 cells were metabolically labelled with [^{32}P] orthophosphate, serum deprived and then serum stimulated in the presence or absence of the compound. The lipids were extracted and subjected to TLC and the bis-phosphorylated $PtdIns(3,5)P_2$ containing spot confirmed by HPLC. Endogenous levels of $PtdIns(3,5)P_2$ are normally very low but increase dramatically upon serum, insulin, hyperosmotic stress and other stimuli. In the presence of 800nM compound (1), $PtdIns(3,5)P_2$ production was decreased by 80% (Figure 1). All other phosphoinositides identified were not substantially altered, although $PtdIns(4,5)P_2$ displayed a fall of 20%.

Example 4: Compound (1) does not affect PKBSer473 phosphorylation

The *in vitro* properties and the *in vivo* effects on lipid metabolism indicate that compound (1) shows some selectivity for the $PtdIns(3,5)P_2$ pathway. The *in vivo* specificity of compound (1) was addressed further by exploring its impact on PKB phosphorylation as an indicator of PI3Kinase/$PtdIns(3,4,5)P_3$ signalling. As the preferred substrate for class I PI3kinases, this addresses whether the modest decrease in $PtdIns(4,5)P_2$ noted above has any impact on this pathway. Under low serum conditions PKB Ser473 phosphorylation in NIH3T3 cells was undetectable (Figure

2A). Serum stimulation induced extensive phosphorylation of Ser473 (Figure 2B). As expected 10μM LY204002 blocked Ser473 phosphorylation and rapamycin, which together with its cellular binding partner FKBP12 inhibits mTOR, had no effect on Ser473 phosphorylation (Figure 2A). In the presence of 800nM compound (1) Ser473 phosphorylation was completely unaffected indicating that at this concentration the compound had no impact on this PI3Kinase pathway (Figure 2A). By contrast with the derivative compound (2) (Figure 2B), phosphorylation of PKB at Ser473 was markedly decreased at 30nM and was completely abolished by 300nM. Thus at 800nM compound (1) displays selectivity towards the p235 pathway.

Example 5: Compound (1) induces the formation of large swollen vesicles

In S. cerevisae carrying mutations in the fab1 gene, a large poorly acidified vacuole is formed, due to the failure to synthesise PtdIns(3,5)P_2. In fibroblasts introduction of a kinase dead mutant of p235 has been shown to alter vesicle morphology. Upon treatment of a variety of cell types including MEF, MDCK, MCF10A, Cos7 and NIH3T3 with compound (1), it was apparent that large vesicles were forming. The size and rate of formation was both time and concentration dependent. A time lapse film of NIH3T3 cells treated for 2 hours with compound (1) and then filmed for a further 2 hours after withdrawal of the drug showed that the formation of swollen vesicles is rapid and is clearly visible after 35min. Indeed, the beginnings of vesicle swelling is virtually immediate following drug treatment and can be visualised both by light and electron microscopy. The presence of compound (1) does not inhibit cell division and, at this concentration of drug, growth curves for NIH3T3 cells over 7 days appear substantially the same as in its absence. Withdrawal of compound (1) results in reversion of the swollen vesicle phenotype with similar kinetics as their formation, such that within 2 hours the cells have reverted to their normal morphology.

Analysis of the ultra-structure of the swollen vesicles by electron microscopy reveals a double limiting membrane. The lumenal content appears diverse and

changes with time of treatment. The accumulated lumenal vesicles include endocytic membrane bound vesicles, very electron dense vesicles (presumed lysosomes/dense core lysosomes), electron dense cytoplasmic material and multilamellar whorls. With increased time of treatment the lumenal space expands and any lumenal vesicles appear confined to the periphery, indeed some swollen vesicles show no lumenal vesicles at all.

Example 6: SiRNA knockout of p235 and rescue of the compound (1) phenotype with Fab1

To further address the specificity of compound (1) inhibition of p235 and the formation of the swollen vesicle phenotype we used two approaches. The first was to design siRNA oligonucleotides to p235 in order to knock down its mRNA and thereby its protein production and activity. Four sequences were selected, two from the N-terminal region and two from the C-terminal region. Introduction of all four caused changes in vesicle formation, however the most potent siRNA found corresponded to cDNA bases 4120-4140. Transfection of siRNA4120-4140 into NIH3T3 cells caused extensive vesicle swelling (Figure 3A), as seen in compound (1) treatments.

In a second approach to confirm specificity use was made of the finding that the yeast p235 homologue, Fab1, was insensitive to compound (1) inhibition (see Table 1). NIH3T3 cells were transfected with an empty plasmid or one containing Fab1. These cells were then treated with compound (1) and fixed. The cells were examined under 63X magnification and in several random fields the number of vesicles greater than 0.5μM in diameter was counted and averaged for the number of cells used in the vesicle counting. This was performed for control cells and the Fab1 transfected cells. The data indicates that expression of the yeast protein Fab1 is able to rescue the effects of compound (1) with an apparent 50-60% reduction in the number of swollen vesicles (Figure 3B). Furthermore the size of the vesicles still present in the Fab1 transfected cells was markedly reduced. It is likely that the

ectopically expressed yeast protein is not appropriately regulated in mammalian cells (the converse has been established) consistent with the partially penetrant rescue. The response to the siRNA and the rescue with Fab1 indicates very strongly that the target for compound (1) action is p235 and PtdIns(3,5)P_2 production.

Example 7: Characterisation of the swollen vesicles induced by compound (1)

To analyse the origins and nature of the vesicle compartment that was being expanded by inhibition of p235, was examined the localisation of a number of markers for early endosomes, late endosomes, the Golgi network, endoplasmic reticulum and the substrate lipid PtdIns3P. Golgi markers such as GM130 and p230 were not found on the swollen vesicle membrane nor on any intralumenal vesicle. Likewise ER markers p62, p115 and calnexin did not appear to be localised to these large vesicles. These results indicate that Golgi and ER do not contribute directly to the extensive accumulation of dilated vesicles. The most likely origin is the endocytic pathway. NIH3T3 stained for EEA1 showed a typical punctate cytoplasmic distribution and treatment with 800nM compound (1) drastically alters this distribution. Under these conditions, EEA1 was found concentrated on the surface of the enlarged vesicular structures and to a certain extent in lumenal vesicles. EEA1 distribution was observed to be limited to the smaller sized vesicles, covering their entire surface and generally absent from the larger vesicles (>5μM). Transient transfection of a GFP tagged tandem fyve domain from Hrs [stenmark] was employed to track the PtdIns3P content of cellular membranes. Co-staining for EEA1 showed not only co-localisation of this protein to areas of PtdIns3P concentration, but that EEA1 can be found concentrated in subdomains on the surface of the swollen vesicle. It was also noted that a subpopulation of vesicles was negative for both the Hrs-fyve domain and EEA1.

Published work with the p235K1831E kinase dead mutant and CIManose-6-phosphate receptor (CI-Man-6-P-Rec) indicates that the site of action of p235 may be on the functioning of the late endosome/multivesicular body (MVB). Cycling

NIH3T3 cells stained for CI-Man-6-P-Rec displayed a defined concentrated perinuclear distrubution on one side of the nucleus. Treatment with 800nM compound (1) did not substantially alter this, save that a relatively small number of enlarged vesicles in the same region stained positive for CI-Man-6-P-Rec. The majority of the enlarged vesicles towards the periphery of the cell and in other areas of the perinuclear region were negative for this marker suggesting that they had lost the CI-Man-6-P-Rec or more likely they were derived from vesicles of an earlier compartment in the endocytic pathway. These observations were reinforced with the co-detection of PtdIns3P using the GFP-Hrs-tandem fyve domain. Co-localisation was seen in the defined perinuclear region but clear separation existed in the other perinuclear areas.

For the vesicles to achieve their inflated size and to maintain their integrity suggests involvment of cytoskeleton and microtubules. Staining of NIH3T3 cells with phalloidin-texas red revealed that the swollen vesicles are sheathed in actin filaments. Formation of these large vesicles is also microtubule dependent. Both vinblastin and nocodazole inhibit their formation indicating that dynamic microtubule-dependent fusion events are likely to be involved.

EM micrographs clearly show the hybrid nature of the compound (1)-induced swollen vesicles. As pointed out above, the lumenal content appears diverse ranging from multiple internal vesicles to those with no observable content. To address the nature of the luminal vesicles and to ask whether in the presence of compound (1) there was still access to the lumen of the swollen vesicle, either by vesicle engulfment, invagination of the endosomal membrane and/or fusion, we used fluorescent tracker dyes. Lucifer yellow in the cell medium was used to track the endocytic pathway. Following compound (1) treatment clear fluorescence was observed within the lumen. The Lucifer yellow was always found confined to membrane-bounded structures within the swollen lumen, the bulk volume of the vesicle did not appear to fluoresce in any of the multiple samples examined. However, access to the lumen via membrane bounded structures was possible whether

the NIH3T3 cells were pre-treated with compound (1) and then exposed to Lucifer yellow or if the cells were pre-loaded with Lucifer yellow and then treated with the test compound. The Lucifer yellow stained intra-lumenal vesicles were generally relatively small and found close to the periphery of the swollen vesicle. Occasionally larger areas of Lucifer yellow were observed which corresponded to optically dense regions in the swollen vesicles. These data implied that both invagination and engulfment events were still occurring in the presence of the drug. To account for the massive increase in the surface area of the swollen vesicles, fusion events must also be occurring.

Tracking the distribution of EGF-receptors in both Cos-7 and NIH3T3 cells after EGF stimulation in the presence of 800nM compound (1) revealed not only that endocytic vesicles are found in the lumen, but also that the outer membrane of a sub-population of swollen vesicles become highly enriched for EGF-receptors.

It is known from yeast deficient in Fab1p that the enlarged vacuole that is formed is poorly acidified. Employing lysotracker, NIH3T3 cells pre-treated with compound (1) were incubated with the dye for 30min. Like the yeast Fab1p deletion mutants, the lumen of the swollen vesicles were very poorly acidified if at all. However surprisingly, some of the intra-luminal vesicles that can be seen in the phase contrast image were strongly fluorescent, demonstrating that acidic vesicles can enter the lumen. Two possibilities exist that are not mutually exclusive. Firstly, the endocytic luminal vesicles may acidify post-entry and secondly it is possible that existing acidified vesicles may be engulfed by the swollen vesicles. These data together show that there is continual access to the outer membrane of the swollen vesicles and to their luminal space and implies that compound (1) is inhibiting recycling of membranes and exit from the compartment.

In the phase contrast images, large areas of dense amorphous material were occasionally seen inside the swollen vesicles. The appearance of these areas suggested a phagocytic process had taken place. Autophagocytosis is a characteristic response to amino acid deprivation and serves to provide these essential metabolites

through recycling non-essential cellular proteins. To assess whether autophagasomal activity contributes material to the swollen vesicles, GFP tagged LC3 was transfected into NIH3T3 cells. LC3 is the mammalian homologue of yeast Apg8p, a protein essential for autophagy in yeast, and has been shown to be associated with autophagosomal membranes. When NIH3T3 cells expressing GFP-LC3 are treated with compound (1) it is apparent that the majority of LC3 accumulates inside the enlarged vesicles. This implies that there is a degree of constitutive autophagy under these conditions and furthermore that the normal autophagic pathway is aberrant. Protein degradation in NIH3T3 cells was stimulated up to 3-fold after 4 hours in amino acid depleted medium, determined by the release of ^{14}C-leucine into the medium. In the presence of compound (1) the amount of ^{14}C-leucine released was reduced by 30%. This was a significant and reproducible reduction and indicates that the autophagocytic breakdown of protein is disrupted by compound (1).

Example 8: Retroviral Production is Inhibited by compound (1)

To test whether interfering with the endosomal compartment might inhibit retrovirus budding in compound (1)- treated cells, TEGH human fibrosarcoma line (TEFLY Mo) producing ecotropic retrovirus containing pBabe-puro were used. Virus titre was measured by infecting NIH3T3 with dilutions of the virus containing medium and selection of the NIH3T3 cells in the presence of puromycin. Remarkably 800nM of compound (1) reduced virus released into the medium by 80% (Figure 4A); this inhibition was dose dependent and with 400nM compound (1) virus titre was reduced by 40-50%. Electron microscopy was used to characterise viral release from the TEGH human fibrosarcoma line treated with or without compound (1). Retrovirus particles inside and outside the cell can be observed. However with compound (1) retrovirus budding is arrested with isolated immature particles remaining connected to the plasma membrane by membrane stalks as well as forming clusters of interconnected virions. Viral particles fail to complete membrane fusion strongly implicating PtdIns3,5P_2 in the budding process.

Example 9 Tablet composition

Tablets, each weighing 0.15 g and containing 25 mg of active compound were manufactured as follows:

Composition for 10,000 tablets

Active compound (250 g)
Lactose (800 g)
Corn starch (415g)
Talc powder (30 g)
Magnesium stearate (5 g)

The active compound, lactose and half of the corn starch were mixed. The mixture was then forced through a sieve 0.5 mm mesh size. Corn starch (10 g) is suspended in warm water (90 ml). The resulting paste was used to granulate the powder. The granulate was dried and broken up into small fragments on a sieve of 1.4 mm mesh size. The remaining quantity of starch, talc and magnesium was added, carefully mixed and processed into tablets.

Example 10: Injectable Formulation

Formulation A	mg
Active compound	200
Hydrochloric Acid Solution 0.1M or	
Sodium Hydroxide Solution 0.1M q.s. to pH	4.0 to 7.0
Sterile water q.s. to	10 ml

The active compound was dissolved in most of the water (35° 40° C) and the pH adjusted to between 4.0 and 7.0 with the hydrochloric acid or the sodium hydroxide as appropriate. The batch was then made up to volume with water and filtered through a sterile micropore filter into a sterile 10 ml amber glass vial (type 1) and sealed with sterile closures and overseals.

Formulation B	mg
Active Compound	125
Sterile, Pyrogen-free, pH 7 Phosphate Buffer, q.s. to	25 ml
Active compound	200 mg
Benzyl Alcohol	0.10 g
Glycofurol 75	1.45 g
Water for injection q.s to	3.00 ml

The active compound was dissolved in the glycofurol. The benzyl alcohol was then added and dissolved, and water added to 3 ml. The mixture was then filtered through a sterile micropore filter and sealed in sterile 3 ml glass vials (type 1).

Example 11: Syrup Formulation

Active compound	250 mg
Sorbitol Solution	1.50 g
Glycerol	2.00 g
Sodium benzoate	0.005 g
Flavour	0.0125 ml
Purified Water q.s. to	5.00 ml

The active compound was dissolved in a mixture of the glycerol and most of the purified water. An aqueous solution of the sodium benzoate was then added to the solution, followed by addition of the sorbital solution and finally the flavour. The volume was made up with purified water and mixed well.

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CLAIMS

1. Use, in the manufacture of a medicament for use as an anti-viral agent, of a compound which inhibits the phosphatidylinositol 3,5-biphosphate pathway.

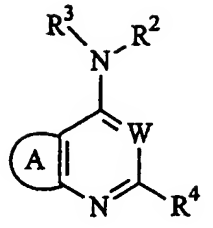
2. Use according to claim 1 wherein the compound is an inhibitor of a phosphatidylinositol 3-phosphate 5-kinase.

3. Use according to claim 2 wherein the phosphatidylinositol 3-phosphate 5-kinase is the p235 enzyme.

4. Use according to claim 1 wherein the compound is an inhibitor of phosphatidylinositol 3-kinase.

5. Use according to claim 4 wherein the phosphatidylinositol 3-kinase is the phosphatidylinositol 3-kinase subtype p110α enzyme.

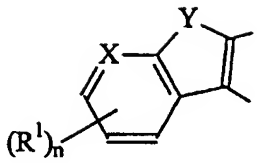
6. Use according to claim 1 wherein the compound is a fused heteroaryl derivative of general formula (I):



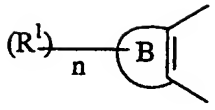
(I)

wherein:

: (a)



or (b)



,

wherein:

B represents a benzene ring, or a 5- or 6-membered monocyclic heteroaryl containing 1 to 2 hetero atoms selected from O, S and N;

R^1 represents lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, aryl which may have one or more substituents, heteroaryl which may have one or more substituents, halogen, $-NO_2$, -CN, a halogenated lower alkyl, -ORb, -SRb, $-SO_2$-Rb, -SO-Rb, -COORb, -CO-Rb, -CONRaRb, $-SO_2$NRaRb, -NRaRb, -NRa-CORb, -NRa-SO_2Rb, -O-CO-NRaRb or -NRaCO-COORb, -CO-a nitrogen-containing saturated

heterocyclic group, -CONRa- lower alkylene-ORb, -CONRa- lower alkylene-NRb, -O-lower alkylene-ORb, -O-lower alkylene-O-lower alkylene-ORb, -O-lower alkylene-NRaRb, -O-lower alkylene-O-lower alkylene-NRaRb, -O-lower alkylene-NRc-lower alkylene-NRaRb, -NRc- lower alkylene-NRaRb, -N(a lower alkylene-NRaRb)$_2$, -CONRa-ORb, -NRa-CO-NRbRc, or -OCORb;

each of R^2 and R^3, which may be the same or different, represents H, lower alkyl, lower alkylene-ORa or lower alkylene-NRaRc, or R^2 and R^3 are combined together with the N atom adjacent thereto to form a nitrogen-containing saturated heterocyclic group as $-NR^2R^3$ which may have one or more substituents;

each of Ra and Rc, which may be the same or different, represents H or lower alkyl;

Rb represents H, lower alkyl, cycloalkyl, aryl which may have one or more substituents or a heteroaryl which may have one or more substituents;

n represents 0, 1, 2 or 3;

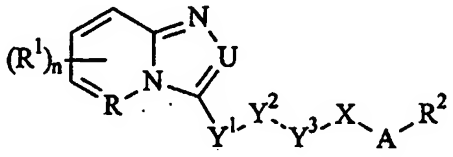
each of W and X, which may be the same or different, represents N or CH;

Y represents O, S or NH; and

R^4 represents H, lower alkyl, lower alkenyl, lower alkynyl, -(aryl which may have one or more substituents), lower alkylene-(aryl which may have one or more substituents), lower alkenylene-(aryl which may have one or more substituents), lower alkynylene-(aryl which may have one or more substituents), -(cycloalkyl which may have one or more substituents), -(cycloalkenyl which may have one or more substituents), lower alkylene-(cycloalkyl which may have one or more substituents), lower alkenylene-(cycloalkyl which may have one or more substituents), lower alkylene-(nitrogen-containing saturated heterocyclic group which may have one or more substituents), lower alkenylene-(nitrogen-containing saturated heterocyclic group which may have one or more substituents), (a heteroaryl which may have one or more substituents), lower alkylene-(heteroaryl which may have one or more substituents), or lower alkenylene-(heteroaryl which may have one or more substituents);

or a pharmaceutically acceptable salt thereof.

7: Use according to claim 1 wherein the compound is an imidazole derivative of general formula (II):



(II)

wherein

R^1 represents H, lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl, cycloalkenyl, halogen, $-NO_2$, -CN, halogenated lower alkyl, $-OR^a$, $-SR^a$, $-SO_2R^a$, $-SOR^a$, $-CO_2R^a$, $-CO-R^a$, aryl, lower alkylene-aryl, -O-lower alkylene-aryl, $-CONR^aR^b$, -CO-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), $-SO_2NR^aR^b$, $-SO_2$-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), $-SO_3H$, a nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group, $-NR^aR^b$, $-CONR^a$-lower alkylene-OR^b, $-CONR^a$-lower alkylene-NR^bR^c, $-CONR^a$-lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), -O-lower alkylene-OR^a, -O-lower alkylene-O-lower alkylene-O^a, -O-lower alkylene-NR^aR^b, -O-lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), -O-lower alkylene-O-lower alkylene-NR^aR^b, -O-lower alkylene-O-lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), -O-lower alkylene-NR^c-lower alkylene-NR^aR^b, -O-lower alkylene-NR^c-lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or

substituted by a lower alkyl group), -OCO-NR^aR^b, -OCO-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), -NR^a-SO_2R^b, -NR^c-lower alkylene-NR^aR^b, -NR^c-lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), -N(lower alkylene-$NR^aR^b)_2$, -N(lower alkylene-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group))$_2$, -$CONR^a$-OR^b, -NR^a-COR^b, -NR^aCO-NR^bR^c, -NR^a-CO-(nitrogen-containing saturated heterocyclic group which is unsubstituted or substituted by a lower alkyl group), or -$OCOR^a$;

R^a, R^b and R^c, which may be the same or different, are H, lower alkyl or aryl;

T is N or CR^{1a};

U is N or CR^3;

n is an integer of 1, 2 or 3;

in Y . . . Y^2 . . . Y^3,

i) . . . represents a single bond on one side and a single or double bond on the other side, Y^1 represents CR^5 or $CR^{5a}R^{5b}$, Y^2 represents N, NH, CR^{4a} or $CR^{4b}R^{4c}$, and Y^3 represents NR^6, CR^{4d} or CR^{4e} R^{4f}, whereas Y^3 represents NR^6 when Y^2 represents CR^{4a} or CR^{4b} R^{4c}, or

ii) Y^1 and Y^3 may be bonded with each other via 2 or 3 atoms and combined with the adjacent Y^2 to form a B ring, wherein the B ring represents a 5- or 6-membered monocyclic heteroaryl ring having 1 to 4 hetero atoms selected from N, S, and O, a nitrogen-containing saturated heterocyclic ring or an aryl ring, whereas said B ring may be substituted by one to two R^4s;

X represents S, SO or SO_2, and X may also represent CO, NR^7 or a methylene group when Y^1 and Y^3 are bonded with each other via 2 or 3 atoms and combined with the adjacent Y^2 to form the B ring;

A represents a direct linkage, lower alkylene, lower alkenylene or lower alkenylene;

R^2 represents lower alkyl which is unsubstituted or substituted by one or more subsituents, lower alkenyl which is unsubstituted or substituted by one or more

subsituents, a lower alkynyl which is unsubstituted or substituted by one or more subsituents, cycloalkyl which is unsubstituted or substituted by one or more subsituents, a cycloalkenyl which is unsubstituted or substituted by one or more subsituents, -N=O, aryl which is unsubstituted or substituted by one or more subsituents, or heteroaryl which is unsubstituted or substituted by one or more subsituents;

R^{1a}, R^3, R^{4a}, R^{4c}, R^{4d}, R^{4e}, R^{4f}, R^{5a} and R^{5b}, which may be the same or different, represent a group defined by R^1, or R^{4b} and R^{4c}, R^{4e} and R^{4f}, or R^{5a} and R^{5b} may be combined with each other to form an oxo group (=O);

R^4 represents a group defined by R^1, or an oxo group (=O); and

R^5, R^6 and R^7, which may be the same or different, represent -H, a lower alkyl which is unsubstituted or substituted by one or more subsituents, lower alkenyl which is unsubstituted or substituted by one or more subsituents, or lower alkynyl which is unsubstituted or substituted by one or more substituents;

or a pharmaceutically acceptable salt thereof.

8. Use according to claim 6 wherein the compound is selected from:

6-amino-3'-(4-morpholinopyrido[3',2':4,5]furo [3,2-d] pyrimidin-2-yl)nicotinanilide;

3-(4-morpholinopyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl) phenol;

and the pharmaceutically acceptable salts thereof.

9. Use according to claim 7 wherein the compound is:

2'-[(6-bromoimidazo[1,2-a]pyridin-3-yl)methylidene]-1',2-dimethyl-5-nitrobenzenesulfonohydrazide

or a pharmaceutically acceptable salt thereof.

10. Use according to any one of the preceding claims wherein the medicament is

for use in preventing or treating an infection caused by a budding virus.

11. Use according to any one of the preceding claims wherein the medicament is for use in preventing or treating an infection caused by a retrovirus.

12. Use according to claim 11 wherein the retrovirus is HTLV-1, HTLV-2 or HIV.

13. A method of treating a patient in need of an anti-viral agent, which method comprises administering thereto a compound which inhibits the phosphatidylinositol 3,5-biphosphate pathway.

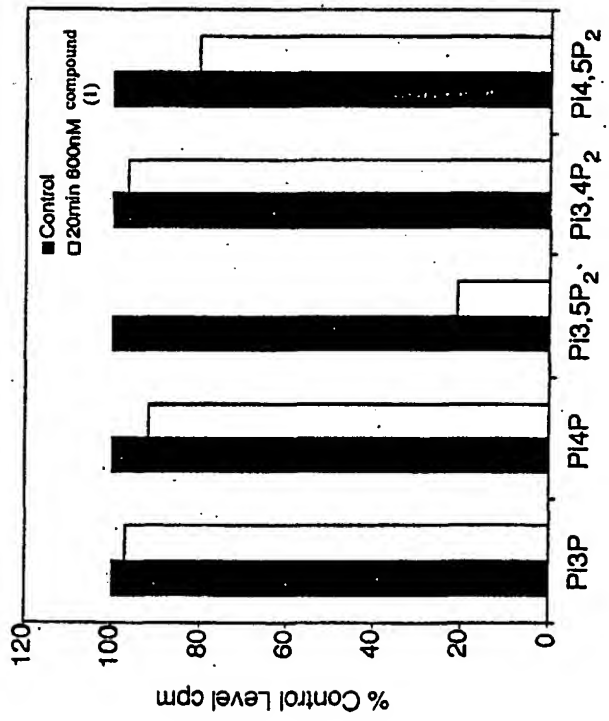

Control
20min 800nM compound (I)
% Control Level cpm
120
100
80
60
40
20
0
PI3P
PI4P
PI3,5P2
PI3,4P2
PI4,5P2

Figure 1

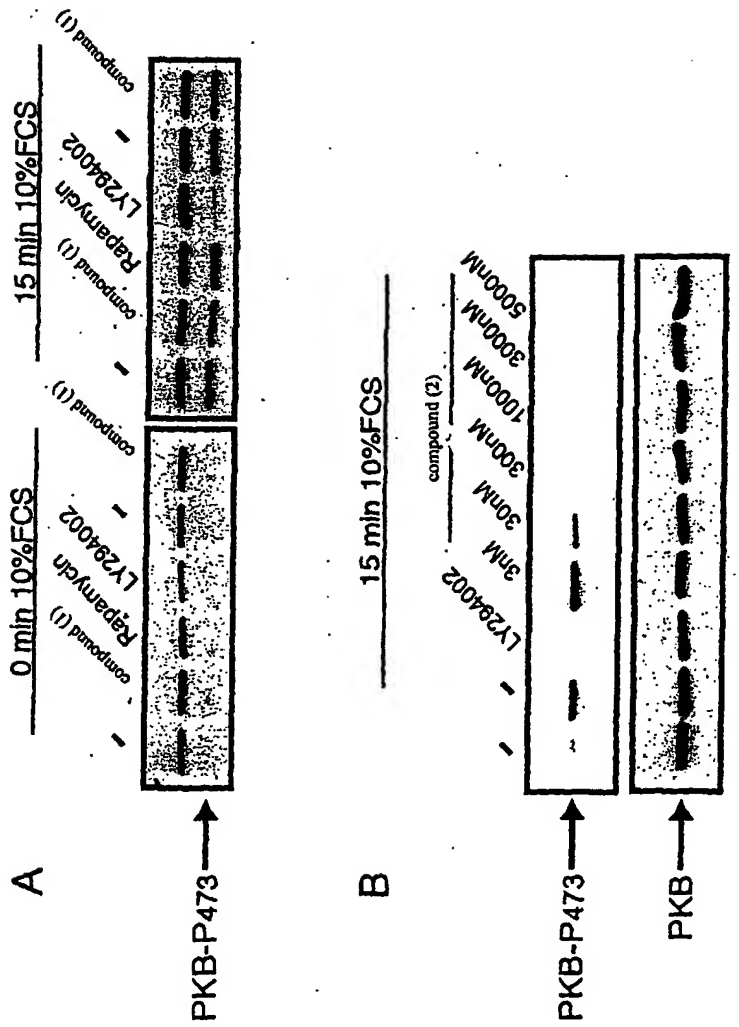
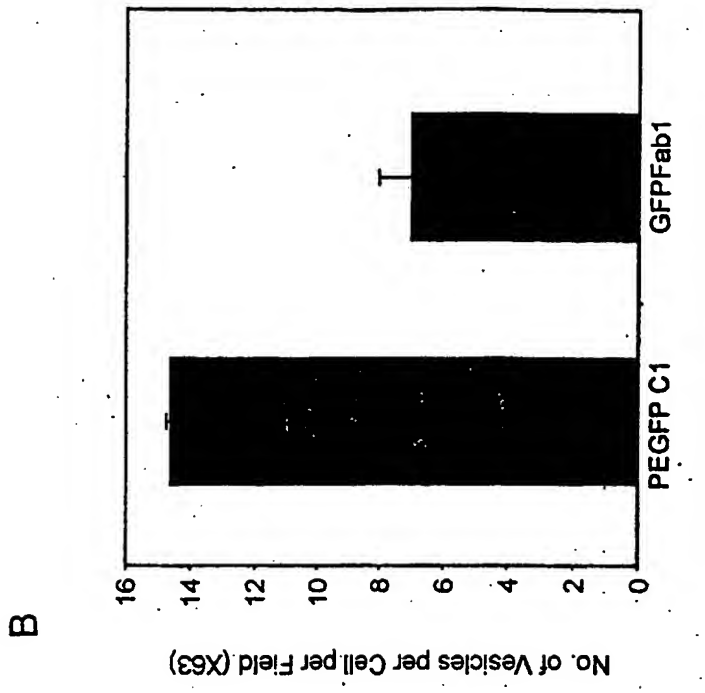

A
0 min 10%FCS
15 min 10%FCS
compound (1)
Rapamycin
LY294002
PKB-P473
B
15 min 10%FCS
compound (2)
LY294002
3nM
30nM
300nM
1000nM
3000nM
5000nM
PKB-P473
PKB

Figure 2



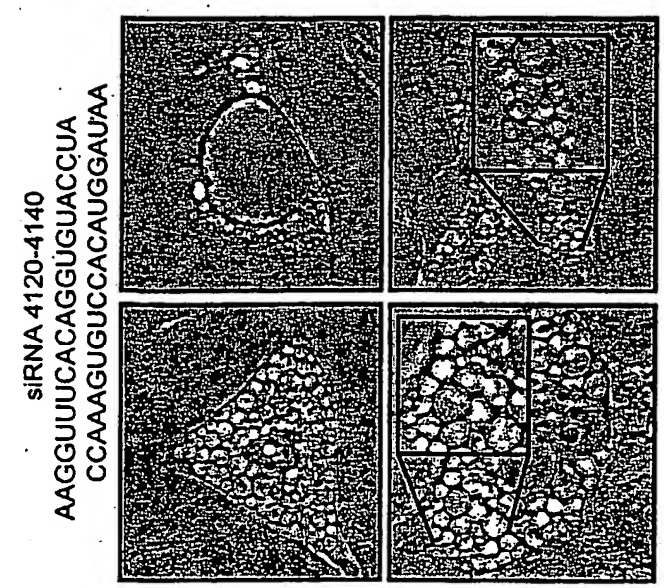


Figure 3

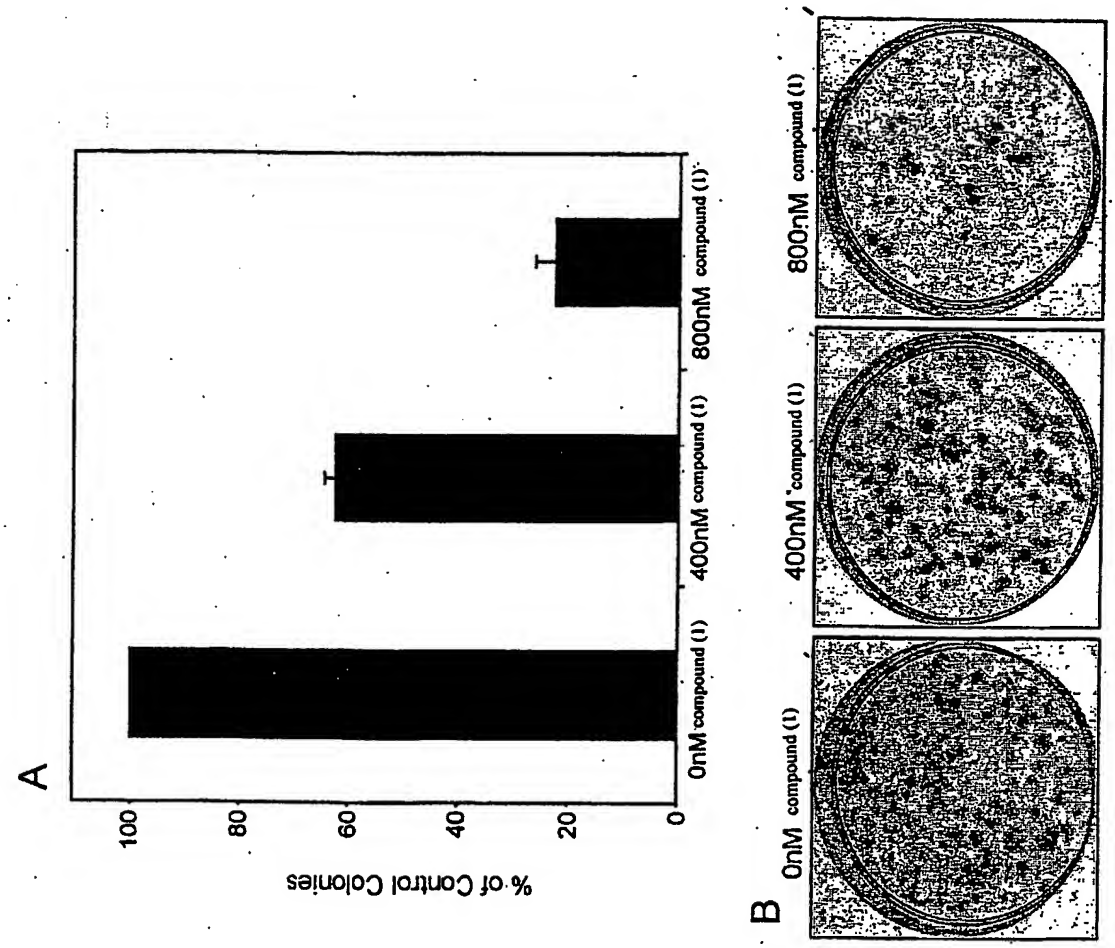


Figure 4